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Feature Articles

Reversal of P-Glycoprotein-associated Multidrug Resistance: The Challenge Continues

Manfred Lehnert

REVERSAL OF P-glycoprotein-associated multidrug resistance, also termed MDR1, has received much attention in recent years. The appeal is obvious. Resistance to cytotoxic therapy has remained a major obstacle to more successful cancer treatment; many of the most frequently used cytotoxic agents are affected by the MDR1 mechanism, including anthracyclines, vinca alkaloids and podophyllotoxines [1, 2]; and there is accumulating evidence to suggest that MDR1 does have clinical relevance in various cancers [3–8].

A number of agents has been identified which are capable of overcoming MDR1 *in vitro* and in animal models. Several comprehensive reviews have recently been published on this subject [9–11]. Briefly, such so-called chemosensitisers (CS) are believed to function by blocking the P-glycoprotein-mediated efflux of the cytotoxic drugs which results in increased intracellular drug accumulation and thus cytotoxicity.

Various CS have been tested in clinical studies, including racemic verapamil, the R-enantiomer of verapamil, cyclosporin A, quinidine, trifluoperazine and tamoxifen [12–20]. Preliminary results have been promising in malignant lymphomas, poor risk acute myeloid leukaemias and multiple myeloma [13, 14, 17]. In solid tumours, however, results generally have been poor. The object of this review is to point to some lessons learned from those studies and to discuss some measures which might be able to narrow the gap between preclinical and clinical effectiveness of CS in the future.

Most of the agents used in clinical studies have been "first-generation" CS such as verapamil or cyclosporin A. These agents originally had been developed for pharmacological effects other than circumvention of MDR1. It thus is not surprising that dose escalation in MDR1 reversal studies has often resulted in serious toxicities [12, 19, 21]. As a result, the plasma levels achieved by CS have usually been well below the concentrations needed for effective MDR1 reversal *in vitro*. More recently, various agents have been specifically developed for overcoming MDR1. Some of these second-generation CS have shown high molar potency in reversing MDR1, e.g. the cyclosporin A analogue PSC-833 [22], SDZ 280–446, a semi-synthetic cyclopeptolide [23], the tiapamil analogue Ro11-22933 [24], the triazineaminopiperidine derivative S 9788 [25] or the dihydropyridine compound B8509-035 [26]. Little is known to date about the maximum tolerated plasma levels of those compounds in humans and their dose-limiting toxicities. Still, some of these agents indeed seem promising and might prove useful for clinical reversal of MDR1.

A novel approach that might turn out to have clinical utility is combined chemosensitisation. The rationale behind this strategy is to increase the therapeutic index of chemosensitisation by combining agents which produce positive interaction in reversing MDR1 but differ in dose-limiting toxicities. Various CS have shown synergism in reversing MDR1 *in vitro*, i.e. verapamil in combination with quinine or cyclosporin A [27, 28]. It should be noted, though, that interaction can be antagonistic between CS, particularly when used in cell lines that express low levels of P-glycoprotein as typically found in clinical tumour specimens [29]. Such observations underscore the need to test each combination of CS in relevant model systems before clinical use. Several clinical studies are currently underway which evaluate various CS combinations, e.g. verapamil and quinine.

Most CS have been described as being promising for clinical use, based on data from standard *in vitro* studies. However, clinical effectiveness has been limited to date. Accordingly, better models are needed to assess potential clinical usefulness of CS. The transgenic mouse model developed at the U.S. National Cancer Institute is one approach which might prove useful in this respect [30]. These animals have been engineered to carry the human *mdr*1 gene in their bone marrow cells, which allows the evaluation of the *in vivo* ability of agents to reverse MDR1 in a rapid and reproducible fashion. Another effort along those lines has been the development of a pharmacologically based *in vitro* model for better estimating the clinical usefulness of CS [31, 32]. In this model, agents are tested in a serum-rich environment at concentrations that can be achieved in human plasma. To further enhance clinical relevance, cell lines are used which exhibit low degrees of MDR1. Standard *in vitro* studies of CS are usually done in medium containing low serum concentrations and the agents are used at doses which are well above the maximum tolerated plasma levels. Typically, cell lines are used which express MDR1 levels which are much higher than is usually found in human cancers. We have found that only a few first-generation CS retain the ability to overcome MDR1 *in vitro* when evaluated in the pharmacologically based model, i.e. cyclosporin A, quinidine and quinine. Such models may prove useful in the future for selecting CS for clinical studies which have a better chance of being effective in reversing MDR1.

For the time being, clinical reversal of MDR1 continues to be an experimental approach. There is no proof to date that adding a CS to chemotherapy does enhance efficacy. For example, data on high-dose infusional verapamil in malignant lymphomas seems the most encouraging at the moment [14]. 18 patients with progressive, drug-refractory lymphoma, who were pretreated with various doxorubicin-containing protocols, received

Correspondence to M. Lehnert, Cancer Research Laboratories, Department C of Internal Medicine, Kantonsspital, CH-9007 St. Gallen, Switzerland.
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CVAD chemotherapy supplemented with infusional verapamil. The CVAD protocol consisted of 1-day cyclosphosphamide, and 4-day continuous infusion doxorubicin and vincristine plus oral dexamethasone. CVAD plus verapamil was able to re-induce remissions in 72% of the patients, including five complete responses. These are no doubt impressive results in this particular patient population. It is unclear, however, to what extent verapamil has contributed to those results. P-glycoprotein expression in lymphoma was analysed in 11 of the patients. 5 of 7 patients (71%) whose tumours expressed P-glycoprotein responded to CVAD plus verapamil. Of the 4 patients who had P-glycoprotein-negative lymphoma, two responded to the treatment, a non-significant difference ($P = 0.47$). Moreover, and most importantly, the patients had not been treated before with CVAD alone, i.e. infusional application of doxorubicin and vincristine. In a similar group of patients with "drug-refractory" lymphoma, similar response rates have been reported for infusional doxorubicin, vincristine and etoposide plus intravenous cyclophosphamide and oral prednisone, without the addition of a CS [15]. Recently published *in vitro* studies using P-glycoprotein-positive colon cancer cell lines have demonstrated a significant reduction in doxorubicin resistance following exposure of cells to drug over several days as compared with several hours [33]. These findings suggest that patients need to be treated with the identical cytotoxic regimen as used in conjunction with a CS to being able to unequivocally assess the CS effects on chemotherapy. This can be achieved by either interpatient comparison in prospective, randomised trials or intrapatient comparison, i.e. treatment of patients with chemotherapy alone followed by the same chemotherapy supplemented with a CS.

It is to be emphasised that supplementing chemotherapy with CS bears the potential of enhancing the toxicity of cytotoxic agents. P-glycoprotein is expressed by many normal cells [34]. It has always been a concern that the use of agents which are capable of blocking P-glycoprotein function may increase the toxicity of cytotoxic drugs on those particular tissues [35]. Most clinical trials have not confirmed these concerns. However, the CS have usually failed to yield effective plasma levels. Novel agents with higher molar potency in inhibiting P-glycoprotein function or which can be given at higher doses might well turn out to substantiate those fears. Recent phase I studies have shown the addition of escalating doses of cyclosporin A to etoposide or vinblastine to result in a progressive increase in the area under the plasma disappearance curve of the cytotoxic agents, associated with increased myelotoxicity [36–38]. It has been reasoned that cyclosporin A at higher concentrations may inhibit hepatic and/or renal excretion of etoposide and vinblastine by blocking P-glycoprotein function in liver and kidney cells, respectively. These observations emphasise the need to study the effects of CS on the pharmacokinetics of cytotoxic agents. Without such studies, proper interpretation of the mechanism(s) responsible for enhanced chemotherapy activity by addition of a CS seems difficult.

Multidrug resistance can result from various molecular mechanisms, i.e. MDR1, reduced amounts or function of topoisomerase II, changes in the glutathione system, and others [39]. Moreover, resistance to drugs such as etoposide, doxorubicin or mitoxantrone can be conferred by either of those mechanisms. Because CS are only able to reverse MDR1, proper interpretation of therapeutic results requires information on MDR1 expression in tumour cells. The majority of published CS studies lack such information. In most haematological malignancies, cancer cells can be readily obtained for analysis of MDR1 expression. Obviously, this is much more difficult in patients with metastatic solid tumours. Still, every effort should be made to analyse MDR1 expression in cancer cells of patients who are planned to receive CS for overcoming clinical drug resistance.

There is now preliminary evidence to suggest that the concept of chemosensitisation can function not only *in vitro* and in animal models but also in cancer patients. However, effective clinical reversal of MDR1 is a complex and difficult task and much work is left to optimise this strategy and to determine whether effective MDR1 reversal will indeed be able to improve efficacy of chemotherapy.

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Oncogenes and Tumour Suppressor Genes in Transgenic Mouse Models of Neoplasia

Deborah J. Fowlis and Allan Balmain

INTRODUCTION

THE STUDY of the molecular mechanisms of carcinogenesis has been greatly enhanced in recent years by the advent of transgenic mouse technology. It is now possible to introduce cloned genes directly into the germ line of mice in such a way that the genes are inherited in a stable Mendelian fashion. This approach enables the study of the *in vivo* function of genes thought to play important roles in the control of cell growth, development and differentiation. This short review will concentrate on the application of these techniques to investigate mechanisms of neoplastic development in specific tissues.

It is now acknowledged that proto-oncogenes, present in all normal cells, play a pivotal role in many human and animal cancers after they have undergone a genetic alteration leading to aberrant expression or function of the gene product [1]. However, from studies of human tumours it is difficult, if not impossible, to determine whether such changes are the cause or consequence of neoplastic development. These questions can only be addressed using animal model systems in which the various steps of tumour initiation and progression can be reproduced, either by mutation of the appropriate genes using chemical carcinogens [2, 3], or by direct introduction of mutant genes into the germline of mice [4]. Transgenic mice offer the possibility of investigating the effects of proto-oncogenes or their activated counterparts *in vivo*, when expressed using their

Correspondence to A. Balmain.
D.J. Fowlis is at the Duncan Guthrie Institute of Medical Genetics, Yorkhill Hospital, Glasgow; and A. Balmain is at the CRC Beatson Laboratories, Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, U.K.
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